



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION,
PESTICIDES
AND TOXIC
SUBSTANCES

MEMORANDUM

DATE: July 16, 2008

SUBJECT: Efficacy Review for Aseptrol S10-Tab; EPA Reg. No. 70060-19;
DP Barcode: D352421

FROM: Lorilyn M. Montford
Efficacy Evaluation Team
Antimicrobials Division (7510P)

THRU: Dr. Tajah Blackburn, Team Leader
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APPLICANT: BASF Catalysts LLC
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FORMULATION FROM LABEL:

<u>Active Ingredient(s)</u>	<u>% by wt.</u>
Sodium Chlorite.....	20.8%
Sodium Dichloroisocyanurate dihydrate.....	7.0%
Inert Ingredients.....	72.2%
Total.....	100.0%

I BACKGROUND

The product, Aseptrol S10-Tab (EPA Reg. No. 70060-19), is an Agency approved sterilant, disinfectant (bactericide, tuberculocide, virucide), and sanitizing rinse for use on hard, non-porous surfaces in industrial, institutional, commercial, animal care, and hospital or medical environments. The label makes no claims with regard to product effectiveness in the presence of hard water or organic soil load. The applicant requested to amend the product label to include claims for effectiveness as a disinfectant against Feline panleukopenia virus, Pseudorabies, Swine influenza A virus, and Transmissible gastroenteritis virus; and as a food contact sanitizer at 25 ppm against *Listeria monocytogenes*. Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated April 28, 2008), EPA Form 8570-1 (Application for Pesticide), EPA Form 8570-34 (Certification with Respect to Citation of Data), six studies (MRID 474157-02 through -07), Statements of No Data Confidentiality Claims for all six studies, and the proposed label.

II USE DIRECTIONS

The product is designed for disinfecting hard, non-porous surfaces such as bio-safety hoods, cages, coops, counters, crates, floors, instruments, kennels, sinks, tiles, utensils, and walls. The product also is designed for sanitizing hard, non-porous, food-contact surfaces and utensils. The proposed label indicates that the product may be used on hard, non-porous surfaces including: ceramic, fiberglass, glass, metal, plastics (polyester, polypropylene, polystyrene, polyvinyl chloride), and stainless steel. Directions on the proposed label provided the following information regarding preparation and use of the product:

As a disinfectant against specific viruses: In a clear plastic pail, place eight 1.5 gram tablets per gallon of water to make a 200 ppm ClO_2 solution. Prepare in a well ventilated area. Wait 10 minutes for the 1.5 gram tablets to completely dissolve. Apply the use solution to pre-cleaned surfaces and instruments thoroughly wetting surfaces with a cloth, mop, sponge, or coarse sprayer, or by immersion. Treated surfaces must remain wet for 10 minutes. Allow to air dry.

As a sanitizing rinse on food contact surfaces: In a clear plastic pail, place one 1.5 gram tablet per gallon of water to make a 25 ppm ClO_2 solution. Prepare in a well ventilated area. Wait 10 minutes for the 1.5 gram tablet to completely dissolve. Remove gross food particles and soil by a pre-flush, pre-scrape, or pre-soak. Thoroughly wash or flush surfaces with a good detergent or compatible cleaner, followed by a potable water rinse. Apply the use solution to pre-cleaned surfaces thoroughly wetting surfaces with a cloth, mop, sponge, coarse sprayer, or by immersion. Treated surfaces must remain wet for at least 60 seconds. Drain and allow to air dry. Do not rinse.

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products as Disinfectants Method (for spray disinfectants) must be used. To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of 2 different product lots of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. Separate studies are required for each virus. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level.

Sanitizing Rinses (For Previously Cleaned, Food Contact Surfaces)

Sanitizing rinses may be formulated with iodophors, mixed halides, or chlorine-bearing chemicals. The effectiveness of such sanitizing rinses for previously cleaned, food contact surfaces must be substantiated by data derived from the AOAC Available Chlorine Germicidal Equivalent Concentration Method. Data from one test on each of 3 product samples, representing 3 different product lots, one of which is at least 60 days old against *Salmonella enterica* (formerly *Salmonella choleraesuis*) are required. Test results must show product concentrations equivalent in activity to 50, 100, and 200 ppm of available chlorine. The reference standard is sodium hypochlorite.

Note: The organism, *Salmonella typhi*, is no longer available for efficacy testing of antimicrobial products. The surrogate organism, *Salmonella enterica*, is to be used as the standard for the *Salmonella* genus.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 474157-02 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Transmissible Gastroenteritis" for Aseptrol S-Tab 10, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 2, 2008. Project Number A05578.

This study was conducted against Transmissible gastroenteritis virus (strain not specified; obtained from the University of Minnesota), using porcine fetal testis cells (ST cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 0808-4287 and 5319) of the product, Aseptrol S-Tab 10, were tested according to ATS Labs Protocol No. ENG01102907.TGE (copy provided). Use solutions were prepared by adding one (1) 1.5 g tablet of the product and 600 mL of 400 ppm AOAC synthetic hard water (titrated at 398 ppm; 202-225 ppm ClO_2 solutions). The use solutions were allowed to stand at room temperature for 5 minutes, swirled to mix, and stored in the dark. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 52% relative humidity. For each lot of product, separate dried virus films were sprayed (three sprays) with the use solution at a distance of 6-9 inches from the carrier surface. The virus films were

exposed to the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. Each virus-disinfectant mixture was immediately passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. ST cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

2. MRID 474157-03 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Feline Panleukopenia virus" for Aseptrol S-Tab 10, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 2, 2008. Project Number A05579.

This study was conducted against Feline panleukopenia virus (Strain Philips-Roxane; ATCC VR-648), using feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 0808-4287 and 5319) of the product, Aseptrol S-Tab 10, were tested according to ATS Labs Protocol No. ENG01102907.FPLV (copy provided). Use solutions were prepared by adding one (1) 1.5 g tablet of the product and 635 mL of 400 ppm AOAC synthetic hard water (titrated at 394-406 ppm; 194-209 ppm ClO₂ solutions). The use solutions were allowed to stand at room temperature for 5 minutes, swirled to mix, and stored in the dark. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (three sprays) with the use solution at a distance of 6-9 inches from the carrier surface. The virus films were exposed to the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. Each virus-disinfectant mixture was immediately passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 14 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. On the final day of incubation, a hemagglutination assay was performed on the cultures using swine red blood cells at 2-8°C. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

3. MRID 474157-04 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Pseudorabies" for Aseptrol S-Tab 10, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 2, 2008. Project Number A05577.

This study was conducted against Pseudorabies (Strain Aujeszky; ATCC VR-135), using feline kidney cells (CRFK cells; ATCC CCL-94; propagated in-house) as the host system. Two lots (Lot Nos. 0808-4287 and 5319) of the product, Aseptrol S-Tab 10, were tested according to ATS Labs Protocol No. ENG01102907.PSRV (copy provided). Use solutions were prepared by adding one (1) 1.5 g tablet of the product and 635 mL of 400 ppm AOAC synthetic hard water

(titrated at 394-406 ppm; 194-209 ppm ClO₂ solutions). The use solutions were allowed to stand at room temperature for 5 minutes, swirled to mix, and stored in the dark. The stock virus culture contained 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (three sprays) with the use solution at a distance of 6-9 inches from the carrier surface. The virus films were exposed to the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. Each virus-disinfectant mixture was immediately passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. CRFK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

4. MRID 474157-05 "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces, Virus: Swine Influenza A" for Aseptrol S-Tab 10, by Kelleen Gutzmann. Study conducted at ATS Labs. Study completion date – January 2, 2008. Project Number A05580.

This study was conducted against Swine influenza A virus (Strain A/Swine/Iowa/15/30; ATCC VR-333), using Rhesus monkey kidney cells (RMK cells; obtained from ViroMed Laboratories, Inc., Cell Culture Division; maintained in-house) as the host system. Two lots (Lot Nos. 0808-4287 and 5319) of the product, Aseptrol S-Tab 10, were tested according to ATS Labs Protocol No. ENG01102907.SFLU (copy provided). Use solutions were prepared by adding one (1) 1.5 g tablet of the product and 635 mL of 400 ppm AOAC synthetic hard water (titrated at 394-406 ppm; 194-209 ppm ClO₂ solutions). The use solutions were allowed to stand at room temperature for 5 minutes, swirled to mix, and stored in the dark. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of separate sterile glass Petri dishes. The virus films were air-dried for 20 minutes at 20.0°C at 50% relative humidity. For each lot of product, separate dried virus films were sprayed (three sprays) with the use solution at a distance of 6-9 inches from the carrier surface. The virus films were exposed to the use solution for 5 minutes at 20.0°C. Following exposure, the plates were scraped with a cell scraper to re-suspend the contents. Each virus-disinfectant mixture was immediately passed through a Sephadex column, and diluted serially in Minimum Essential Medium supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL amphotericin B. RMK cells in multi-well culture dishes were inoculated in quadruplicate with 0.1 mL of the dilutions. The cultures were incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂. The cultures were scored periodically for 7 days for the presence or absence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included those for dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

5. MRID 474157-06 "AOAC Available Chlorine in Disinfectants, Test Organism: *Listeria monocytogenes* (ATCC 19111)" for S Tab10, by Becky Lien. Study conducted at ATS Labs. Study completion date – September 29, 2006. Project Number A04281.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). One lot (Lot No. 0407-22) of the product, S Tab10, was tested using the AOAC Chlorine (Available) in Disinfectants Germicidal Equivalent Concentration Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A use solution containing 25 ppm total available ClO_2 was prepared. One (1) 1.5 g tablet of the product was added to 2 L of 400 ppm AOAC synthetic hard water (titrated at 402 ppm). The use solution was allowed to stand at room temperature for 15 minutes and was then swirled to mix. A 416 mL aliquot of this solution was further diluted to a total volume of 1 L with 400 ppm AOAC synthetic hard water. This dilution was verified by titration to be 30.7 ppm ClO_2 . A second dilution was made by adding 100.0 mL of 400 ppm AOAC synthetic hard water, resulting in a 27.3 ppm ClO_2 solution. NaOCl control solutions of 200, 100, and 50 ppm available chlorine were prepared. A 0.05 mL aliquot of the test suspension was added to each of the NaOCl control solutions and the use solution at 20.0°C. After one minute, a loopful (i.e., 0.05 mL) of each culture-solution mixture was transferred into Lethen Broth with 0.1% sodium thiosulfate. Each tube was then challenged with an additional 0.05 mL aliquot of the test suspension 30 seconds after subculturing. This procedure was repeated for a total of 10 subcultures for the use solution and each NaOCl control solution. All subcultures were incubated for 46 hours at 35-37°C and then stored for 3 days at 2-8°C. Following incubation and storage, the subcultures were examined for the presence or absence of visible growth. Controls included initial suspension counts, purity, viability, and sterility. The neutralization confirmation control was waived because sufficient growth in the test system and chlorine control subcultures was observed.

6. MRID 474157-07 "AOAC Available Chlorine in Disinfectants, Test Organism: *Listeria Monocytogenes* (ATCC 19111)" for S Tab10, by Becky Lien. Study conducted at ATS Labs. Study completion date – November 7, 2006. Project Number A04399.

This study was conducted against *Listeria monocytogenes* (ATCC 19111). One lot (Lot No. 0808-4287) of the product, S Tab10, was tested using the AOAC Chlorine (Available) in Disinfectants Germicidal Equivalent Concentration Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. The product lot was at least 60 days old at the time of testing. A use solution containing 25 ppm total available ClO₂ was prepared. One (1) 1.5 g tablet of the product was added to 2 L of 400 ppm AOAC synthetic hard water (titrated at 404 ppm). The use solution was allowed to stand at room temperature for 15 minutes and was then swirled to mix. A 416 mL aliquot of this solution was further diluted to a total volume of 1 L with 400 ppm AOAC synthetic hard water. This dilution was verified by titration to be 29.8 ppm ClO₂. A second dilution was made by adding 115 mL of 400 ppm AOAC synthetic hard water, resulting in a 25.6 ppm ClO₂ solution. NaOCl control solutions of 200, 100, and 50 ppm available chlorine were prepared. A 0.05 mL aliquot of the test suspension was added to 10 mL of each of the NaOCl control solutions and the use solution at 21.0°C. After one minute, a loopful (i.e., 0.05 mL) of each culture-solution mixture was transferred into Letheen Broth with 0.1% sodium thiosulfate. Each tube was then challenged with an additional 0.05 mL aliquot of the test suspension 30 seconds after subculturing. This procedure was repeated for a total of 10 subcultures for the use solution and each NaOCl control solution. All subcultures were incubated for 46 hours at 35-37°C. Following incubation, the subcultures were examined for the presence or absence of visible growth. Controls included initial suspension counts, purity, viability, and sterility. The neutralization confirmation control was waived because sufficient growth in the test system and chlorine control subcultures was observed.

V RESULTS

MRID Number	Organism	Results			Dried Virus Count (TCID ₅₀ /0.1 mL)
			Lot No. 0808-4287	Lot No. 5319	
474157-02	Transmissible gastroenteritis virus	10 ⁻¹ to 10 ⁻⁸ dilutions	Complete inactivation	Complete inactivation	10 ^{6.0}
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	
474157-03	Feline panleukopenia virus	10 ⁻¹ to 10 ⁻⁶ dilutions	Complete inactivation	Complete inactivation	10 ^{5.0}
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	
474157-04	Pseudorabies	10 ⁻¹ to 10 ⁻⁹ dilutions	Complete inactivation	Complete inactivation	10 ^{5.75}
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	
474157-05	Swine influenza A virus	10 ⁻¹ to 10 ⁻⁷ dilutions	Complete inactivation	Complete inactivation	10 ^{5.75}
		TCID ₅₀ /0.1 mL	≤10 ^{0.5}	≤10 ^{0.5}	

MRID Number	Organism	Lot or Concentration	Subculture Series									
			1	2	3	4	5	6	7	8	9	10
474157-06	<i>Listeria monocytogenes</i>	Lot 0407-22	0	0	0	0	+	+	+	+	+	+
	<i>Listeria monocytogenes</i>	NaOCl										
		200 ppm	0	0	0	+	+	+	+	+	+	+
		100 ppm	0	0	+	+	+	+	+	+	+	+
474157-07	<i>Listeria monocytogenes</i>	Lot 0808-4287	0	0	0	0	+	+	+	+	+	+
	<i>Listeria monocytogenes</i>	NaOCl										
		200 ppm	0	0	0	+	+	+	+	+	+	+
		100 ppm	0	0	+	+	+	+	+	+	+	+
		50 ppm	0	+	+	+	+	+	+	+	+	+

+ = Growth of organism; 0 = No growth of organism

VI CONCLUSIONS

1. The submitted efficacy data support the use of the product, Aseptrol S10-Tab (i.e., Aseptrol S-Tab 10), as a disinfectant against the following microorganisms on hard, non-porous surfaces in the presence of 400 ppm hard water and a 5% organic soil load for a contact time of 5 minutes as a ~200 ppm ClO₂ solution:

Transmissible gastroenteritis virus
Feline panleukopenia virus
Pseudorabies
Swine influenza A virus

MRID 474157-02
MRID 474157-03
MRID 474157-04
MRID 474157-05

Complete inactivation (no growth) was indicated in all dilutions tested

2. The submitted efficacy data (MRID 474157-06 and -07) support the use of the product, Aseptrol S10-Tab (i.e., S Tab10), as a sanitizing rinse with bactericidal activity against *Listeria monocytogenes* on hard, non-porous surfaces in the presence of 400 ppm hard water for a contact time of 1 minute as a 25 ppm ClO₂ solution. The test substance showed greater efficacy than a 200 ppm NaOCl solution. Neutralization confirmation testing was waived because sufficient growth in the test system and chlorine control subcultures was observed. The viability controls were positive for growth. The purity controls were reported as pure. The sterility controls did not show growth.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Aseptrol S10-Tab, is an effective disinfectant on hard, non-porous, pre-cleaned surfaces against the following microorganisms for a 5-minute contact time as a 200 ppm ClO₂ solution:

Transmissible gastroenteritis
Feline panleukopenia virus
Pseudorabies
Swine influenza A virus

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Aseptrol S10-Tab, is an effective sanitizing rinse on hard, non-porous, pre-cleaned surfaces against *Listeria monocytogenes* for a 1-minute contact time as a 25 ppm ClO₂ solution. This claim is acceptable as it is supported by the submitted data.

3. The following condition (specified in the Agency letter accompanying the last accepted label (dated October 22, 2007)) was not addressed:

- On page 2 of the proposed label, the claim of efficacy against *Pseudomonas aeruginosa* was not removed, per Agency instructions.

4. The following changes are required on the proposed label::

- On page 2 of the proposed label in the table listing organisms and treatment conditions, change "*Listeria moncytogenes*" to read "*Listeria monocytogenes*."
- On page 2 of the proposed label under the directions for preparing a 25 ppm use solution, change "10 minutes for the [1.5 gram][6.0 gram] tab to completely dissolve" to read "10 minutes for the 1.5 gram tab and 15 minutes for the 6.0 gram tab to completely dissolve."
- On page 3 of the proposed label under the "Food-Contact Surface Sanitizer" section, identify the types of surfaces, objects, and items intended for treatment (e.g., floors, walls).
- On page 5 of the proposed label under the "Surface Sterilization" section, change "fiberglass" to read "sealed fiberglass" and change "ceramic" to read "glazed ceramic." Fiberglass and ceramic are porous surfaces.
- On page 5 of the proposed label under the "Surface Sterilization" section, change "Aseptrol Stab-10" to read "Aseptrol S10-Tab."
- On page 5 of the proposed label, reduce the font size of the second set of container disposal instructions to match the font size of the other storage and disposal instructions.

5. The Data Matrix must be revised to identify a MRID number for the efficacy study against Porcine circovirus type 2. This virus was listed on the last accepted label and the proposed label.